

AMENDMENT

Please amend the application, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows:

In the Specification

--The construction of pTIN406, pTIN408 and pTIN414 has been described (Cannon *et al.*, 1996). The 5' LTR of pH3Z and pH4Z contain a CMV promoter at the U3 position and the HIV R and U5 regions. HIVdge was made from HIVgpt (Page *et al.*, 1990) by blunt-ending the Cla I site (829) to create a frameshift mutation. HIVdge was cut with Bgl II and Pst I (473-1414) and inserted into pTIN406. pTIN406 has an LTR structure of CMV, R (HIV) and U5 (MLV). This created a hybrid LTR containing CMV, and R, U5 from HIV called pBS5'. To provide the plasmid with *rev* and RRE the Eco RI/Xho I fragment (5743-8897) was cut from HIVdge1.2 which is a HIVdge derivative containing a deletion from Nde I to Bgl II (6403-7621) and was ~~inserted~~inserting into pBS5' to create pBS5'R. The 3' LTR was provided by inserting the Not I/Xho I fragment of pBS3' into pBS5'R creating pH2. pBS3' was created by a three way ligation of the Xho I/Hind III fragment of pW13, the Hind III/Kpn I fragment of pTIN408 into Bluescript KS+ (Xho I/KpnI). A CMV promoter was inserted into the unique Xho I site of pH2 from pSPCMV (Sal I/Xho I) making pH2CMV. pSPCMV was created by inserting pLNCX (Accession number: M28246) (Pst I/Hind III) into pSP72 (Promega). The β -galactosidase gene was inserted from PTIN414 into pSP72 (Xho I/Sph I) to make pSPlacZ. A Xho I/Sal I digest of pSPlacZ gave the β -galactosidase coding region which was inserted into pH2-CMV to give pH3Z. pH4Z was constructed to create *tat*-deficient vector. The first 50 bp of the *tat*-coding region was removed by replacing EcoRI (5743)I-SpeI fragment in pH3 with EcoRI (5881)-SpeII PCR product amplified using PCR primers DELT5 (5'-CGTGAATTCGCCTAAACTGCTTGTACCA-3') (SEQ ID NO:1) and DELT3 (5'-GAACTAATGACCCCGTAATTG-3') (SEQ ID NO:2) to create pH4. The Nsi I/Spe I fragment from pH4 was inserted into pH3Z to generate pH4Z.

A *vpr* expression plasmid was constructed by PCR amplification of the *vpr* coding region from pNL4.3 (Accession number: U26942) using the following primers: 5' primer GCGAATTCGGATCCACCATGGAACAAGCCCCAGAAGAC (5563-5583) (SEQ ID NO:3) and 3' primer GCGAATTCGGATCCTCTAGGATCTACTGGCTCCATT (5834-5853) (SEQ ID NO:4). This amplicon was cloned into pLIGATOR (R&D Systems). The expression plasmid

C1 pCl-*vpr* was made by inserting the Mlu I and Xho I fragment containing the *vpr* coding region into pCl-Neo (Promega).--
